different conformations in the intact enzyme density, supporting proposed models of conformational flexibility in the stator subunits.

References
[1] A.M. Balakrishna, C. Hunke, G. Grüber, , Acta Crystallogr. Sect. F Struct. Biol. Cryst. Commun. 66 (2010) 324–326.
[2] L.K. Lee, A.G. Stewart, R.A. Donohoe Bernal, D. Stock, Nat. Struct. Mol. Biol. 17 (2010) 373–378.
[3] Balakrishna A M, Hunke C, Grüber G (2012) *J. Mol. Biol.*, in press.

doi:10.1016/j.bbabio.2012.06.037

1P5

The mechanism of inhibition of bovine F_1-ATPase by the inhibitor protein IF_1

John V. Bason, Arjun Thapaliya, Martin G. Montgomery,
Andrew G.W. Leslie, John E. Walker
MRC Mitochondrial Biology Unit, Hills Road, Cambridge, CB2 0XY, UK
E-mail: jbason19@googlemail.com

In the structure of bovine F_1-ATPase inhibited with residues 1-60 of the bovine inhibitor protein IF_1, one inhibitor protein (I1-60) interacts with five of the nine subunits of F_1-ATPase, and I1-60 is bound tightly at the β_{DP}/α_{DP} catalytic interface.[1] Formation of the inhibited complex requires ATP hydrolysis. It has been proposed that the first interaction between the inhibitor and F_1-ATPase is with the β_E-subunit (the most open state), and that the inhibitor becomes entrapped progressively as two ATP molecules are hydrolysed. We have determined a structure that supports this proposal. The enzyme was inhibited with a mutant form of the inhibitor that binds more strongly than the wild-type protein, and in the structure three inhibitor proteins are bound, one at each of the three catalytic interfaces. This structure indicates that the inhibitor enters the open β_E/α_E catalytic interface at a shallow angle relative to the γ-subunit. In the transition to the β_{TP}/α_{TP} catalytic interface this angle steepens. In the transition of the β_{TP}/α_{TP} interface to a β_{DP}/α_{DP} interface additional secondary structure forms at the N-terminus of IF_1 and extensive interactions between this region and F_1-ATPase observed in the previous inhibited structure are established.

References
[1] J.R. Gledhill, M.G. Montgomery, A.G.W. Leslie, J.E. Walker, How the regulatory protein, IF_1, inhibits F_1-ATPase from bovine mitochondria, Proc. Natl Acad. Sci. U.S.A. 104 (2007) 15671–15676.

doi:10.1016/j.bbabio.2012.06.038

1P6

The chloroplast ATP synthase contains critical targets of reactive oxygen species

Felix Buchert, Christoph Forreiter
Max Planck Institute of Biophysics, Max-von-Laue-Str. 3, 60438 Frankfurt am Main, Germany; and Department Chemie-Biologie, Universität Siegen, Adolf-Reichwein-Str. 2, 57068 Siegen, Germany
E-mail: felix.buchert@googlemail.com

In oxygen-rich cellular powerplants, such as chloroplasts and mitochondria, several highly reactive oxygen species (ROS) are continuously produced and affect a broad range of biomolecules. Accordingly, a potential ROS target in these organelles is the F-ATP synthase, a rotating nanomachine. In chloroplasts, the corresponding enzyme generates ATP by using a transmembrane proton gradient established during photosynthesis. Here, it could be shown that ROS exposure of spinach chloroplast F-ATP synthase dramatically decreased enzymatic activity *in situ* and affected chloroplast coupling factor 1 (CF1) *in vitro*. In order to identify critical sites of oxidative modification, a mapping approach on spinach CF1 γ subunit revealed a conserved sulfurous amino acid cluster of putative targets, consisting of γM23, γC89, γM279, and γM282. In previous studies, some of these residues were shown to be involved in energy coupling [1] and 'catch' formation with the β subunit [2]. Analyzing *in vitro*-assembled hybrid F1 after ROS exposure, MgATPase rates of site-directed mutants indicated that the cluster formed a functional target site for hydrogen peroxide and singlet oxygen. However, it could be deduced from the data that additional unknown residues were supposedly involved in the latter reaction. Furthermore, the cluster was tightly integrated in catalytic turnover since mutants varied in MgATPase activity, sulfite stimulation, and chloroplast-specific γ subunit redox regulation. Regarding impacts on catalysis and response to ROS, it could be shown that defined substitutions within the cluster were dominant over others. Taken together, these studies suggest that an already well-studied γ subunit region might also contain an evolutionary conserved target site involved in oxidative damage.

References
[1] R.K. Nakamoto, K. Shin, A. Iwamoto, H. Omote, M. Maeda, M. Futai, Ann. N. Y. Acad. Sci. 671 (1992) 335–344.
[2] J.P. Abrahams, A.G. Leslie, R. Lutter, J.E. Walker, Nature 370 (1994) 621–628.

doi:10.1016/j.bbabio.2012.06.039

1P7

Observation of twisting within the central stalk of EF_OF_1 during catalysis with single molecule FRET spectroscopy

M. Burger, P. Gräber
Institut für Physikalische Chemie, Albert-Ludwigs Universität Freiburg, Germany
E-mail: markus.burger@physchem.uni-freiburg.de

Subunit movements within the H^+-ATPsynthase from *E. coli* (EF_0F_1) are investigated by single molecule spectroscopy during ATP synthesis and hydrolysis. Two cysteines are introduced at positions γT106C and εY114C within the central stalk and two fluorescence dyes ATTO532 (donor) and ATTO610 (acceptor) are used for labeling. The donor and acceptor labeled EF_0F_1 is integrated into liposomes and a transmembrane pH-difference is generated by an acid base transition. Single-pair fluorescence resonance energy transfer is measured in freely diffusing proteoliposomes with a confocal two channel microscope. During ATP synthesis the fluorescence time traces show a continuously change in FRET-efficiency followed by a stepwise change to the initial efficiency. During ATP hydrolysis a similar sequence with opposite direction of the continuous and stepwise change is found. This finding is supposed to show directly the twisting within the central stalk during catalysis.

doi:10.1016/j.bbabio.2012.06.040

1P8

In situ structure of the mitochondrial F_1F_o ATP synthase dimer and its role in shaping membrane morphology

K.M. Davies, C. Anselmi, B. Daum,
J.D. Faraldo-Gómez, W. Kühlbrandt

Max Planck Institute of Biophysics, Department of Structural Biology, Max-von-Laue Str. 3, 60438 Frankfurt am Main, Germany. Max Planck Institute of Biophysics, Theoretical Molecular Biophysics Group and Cluster of Excellence 'Macromolecular Complexes', Max-von-Laue Str. 3, 60438 Frankfurt am Main, Germany
E-mail: karen.davies@biophys.mpg.de

The ATP synthase is a highly conserved nano-machine which regenerates ATP using the energy stored in a trans-membrane electrochemical gradient. Using electron cryo-tomography (cryo-ET) we determined the macromolecular structure and distribution of ATP synthase in mitochondria from fungi, plants and vertebrates. We found that the mitochondrial ATP synthase forms only one type of dimer. The dimers are arranged in approximately straight rows along the edges of lamellar cristae. Sub-tomogram averaging of the yeast dimer at 3.7 nm resolution reveals a two-fold symmetric V-shape structure, with an angle of 86° between monomers. The central and peripheral stalks are well resolved. The dimer interface is located in the membrane between the two peripheral stalks. By cryo-ET of yeast knockout mutants, we show that subunits *e*, *g*, and the first transmembrane helix of subunit 4 are essential for the formation of this dimer. Without dimers, mitochondria lack the highly curved membrane ridges, which are crucial for shaping lamellar cristae of wild type mitochondria. By large-scale, coarse-grained molecular dynamics simulations, we demonstrate that a single ATP synthase dimer causes a local deformation of the lipid bilayer. This deformation is partially relieved by the side-by-side association of several dimers. As the distance between dimers in a row is variable, we conclude that the assembly of ATP synthase dimer rows is driven by this reduction in membrane elastic energy, rather than by direct protein contacts, and that the dimer rows are an essential element of normal mitochondrial morphology.

doi:10.1016/j.bbabio.2012.06.041

1P9

Assembly of the *b* dimer into the *Escherichia coli* ATP synthase requires subunit delta

G. Deckers-Hebestreit, F. Hilbers, K. Friedrich,
B. Herkenhoff-Hesselmann, E. Becker, K. Brandt, R. Eggers
Universität Osnabrück, Fachbereich Biologie/Chemie, Abteilung Mikrobiologie, D-49069 Osnabrück, Germany
E-mail: deckers-hebestreit@biologie.uni-osnabrueck.de

The F_OF_1-ATP-synthase of *E. coli* is composed of different structural and functional entities that couple the translocation of protons across the cytoplasmic membrane to the synthesis or hydrolysis of ATP. To study the integration of subunit *b* into F_OF_1 during assembly, nearest-neighbor analyses were performed using single-subunit knockout mutants GDH1 and GDH2, in which the synthesis of subunit *a* or delta is prevented by insertion of an early stop codon into the corresponding gene. In GDH1, the ATPase activity as well as the amount of F_OF_1 subunits present in membrane vesicles is comparable to wild type, whereas GDH2 showed an approximately sevenfold reduction. Binding of b_2 to its interacting subunits alpha, beta, and *c* was completely impaired in GDH2, but remained intact in GDH1, indicating that the *b* dimer has no association to the assembled F_OF_1 core complex in the absence of delta. Purification of partially assembled F_OF_1 by affinity chromatography *via* His_6-tagged subunit beta demonstrated that the b_2 is not assembled into the enzyme complex in GDH2. Therefore, subunit delta functions as a brace between the *b* dimer and the F_OF_1 core complex enabling a stable assembly.

The preservation of the prefolded subcomplexes of GDH2 in a stand-by mode ready for integration of subunit delta was studied by a time-delayed *in vivo* assembly system. Two expression systems (P_{araBAD} and $T7_{lac}$) were combined, both exhibiting stringent induction/repression conditions. They thus function in opposition thereby assuring that both systems do not interfere with each other. Consequently, a *de novo* biosynthesis of F_OF_1 is prevented. In detail, all structural *atp* genes except *atpH* were expressed under P_{araBAD} control by induction with arabinose. Following synthesis of F_OF_1 subunits except delta during growth, expression was repressed by glucose/D-fucose and the complete degradation of *atp* mRNA was controlled by real-time RT-PCR. The time-delayed expression of *atpH* was subsequently started *in trans* by addition of IPTG. Formation of functionally assembled F_OF_1 was verified by DCCD-sensitive ATPase activity and ATP-driven proton translocation clearly demonstrating that the subcomplexes of F_OF_1-delta remain in a prefolded state ready to integrate subunit delta into the enzyme complex. In summary, subunit delta is absolutely necessary for a functional assembly of the *b* dimer into F_OF_1, whereupon the individual subcomplexes formed remain capable of being integrated.

doi:10.1016/j.bbabio.2012.06.042

1P10

Generation of a mitochondrial membrane potential in trypanosomes in the absence of proton transport: A key role for mutations in the γ subunit that uncouple ATP synthase

Caroline Dewar, Matthew Gould, Sam Dean, Achim Schnaufer
Centre for Immunity, Infection and Evolution, Institute of Immunology and Infection Research, King's Buildings, University of Edinburgh, Edinburgh, EH9 3JT
E-mail: c.dewar-4@sms.ed.ac.uk

The protist parasite *Trypanosoma brucei* is one of the earliest diverging eukaryotes and shows a number of unusual and fascinating mitochondrial features, including a gigantic organellar DNA network (the kinetoplast or kDNA), extensive-posttranscriptional mRNA editing, and dramatic regulation of mitochondrial function. During the mammalian bloodstream stage of the life cycle, ATP is exclusively generated via glycolysis. We showed that the F_1F_o-ATP synthase acts in reverse to maintain the essential mitochondrial membrane potential[1]. In *T. brucei*, ATP synthase subunit 6 (a), an F_O subunit critical for proton transport, is encoded by the kDNA genome, but naturally occurring dyskinetoplastic subspecies, such as *T. evansi*, are able to exist without kDNA. We show that a single amino acid mutation in the *T. evansi* F_1 γ subunit is sufficient to allow viability in the absence of kDNA, a situation reminiscent of petite-negative yeast [2]. This implies that ATP synthase subunit 6 is the only mitochondrial gene product normally required by bloodstream form *T. brucei*. Surprisingly, in the presence of kDNA (and therefore F_O) the mutation makes the cells insensitive to oligomycin, suggestive of uncoupled F_1 and F_o moieties. In *Saccharomyces*, an uncoupled ATP synthase generates a lethal proton leak and selects for deletion of the mitochondrial genome (and thus F_O) to close the leak, but in *T. brucei* kDNA appears to be relatively stable in this situation. We currently examine how these mutations affect the structure and function of the *T. brucei* ATPase using native tandem affinity purification. Affinity-tagged ATPase subunits are being expressed ectopically in cells that have had one γ allele replaced by a mutant version, and in cells with kDNA deleted. High resolution clear-native electrophoresis will be used to isolate and analyse the complexes from mitochondrial preparations with integrity and activity intact. The kinetic properties of